10/672,059

(12) UK Patent Application (19) GB (11) 2 218 904 (13) A

(43) Date of A publication 29.11.1989

(21) Application No 8812653.7

(22) Date of filing 27.05.1988

(51) INT CL⁴
A61K 31/20

(52) UK CL (Edition J)
A5B BJA B180 B26Y B36Y B364 B40Y B401 B406

(56) Documents cited
GB 2142234 A GB 2139889 A GB 2098065 A
GB 2090529 A
J. Nutr. Sci. Vitaminol 30(4), 357-72 (1984)

(58) Field of search
UK CL (Edition J) A5B BHA BJA
INT CL⁴ A61K

(71) Applicant
Renafield Limited

(Incorporated in the United Kingdom)

100 Chalk Farm Road, London, NW1 8EH,
United Kingdom

(72) Inventor
Max Zigerlig

(74) Agent and/or Address for Service
Matthews Haddan & Co
Haddan House, 33 Elmfield Road, Bromley, Kent,
BR1 1SU, United Kingdom

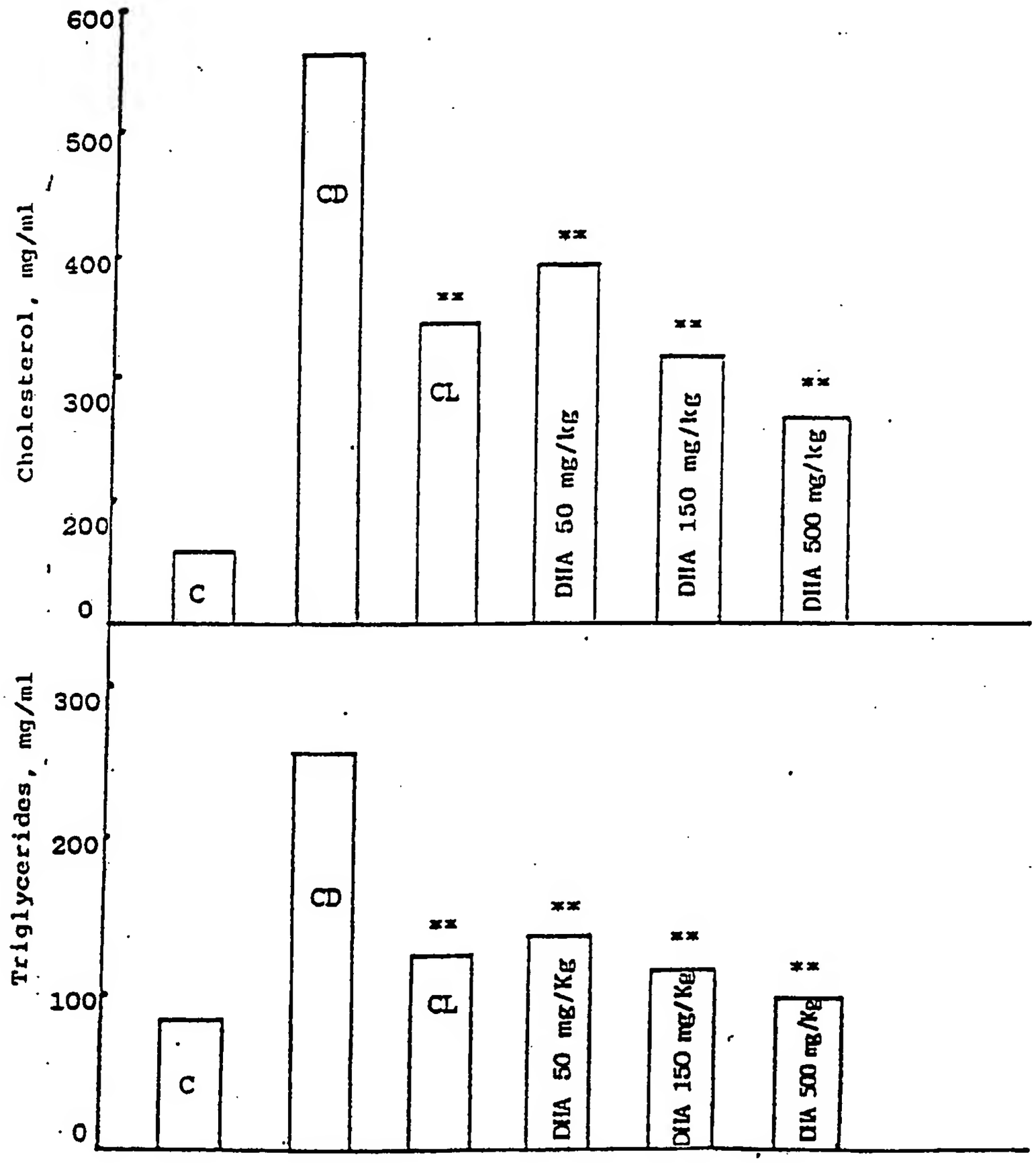
(54) Pharmaceutical composition based on high-concentration esters of docosahexaenoic acid

(57) A pharmaceutical composition contains highly purified, high-concentration docosahexaenoic acid (DHA), prepared in its ester form, and with a suitable addition of tocopherol as anti-oxidant which is used for the prophylaxis and the management of malfunctions, diseases and pathologies.

GB 2 218 904 A

2218904

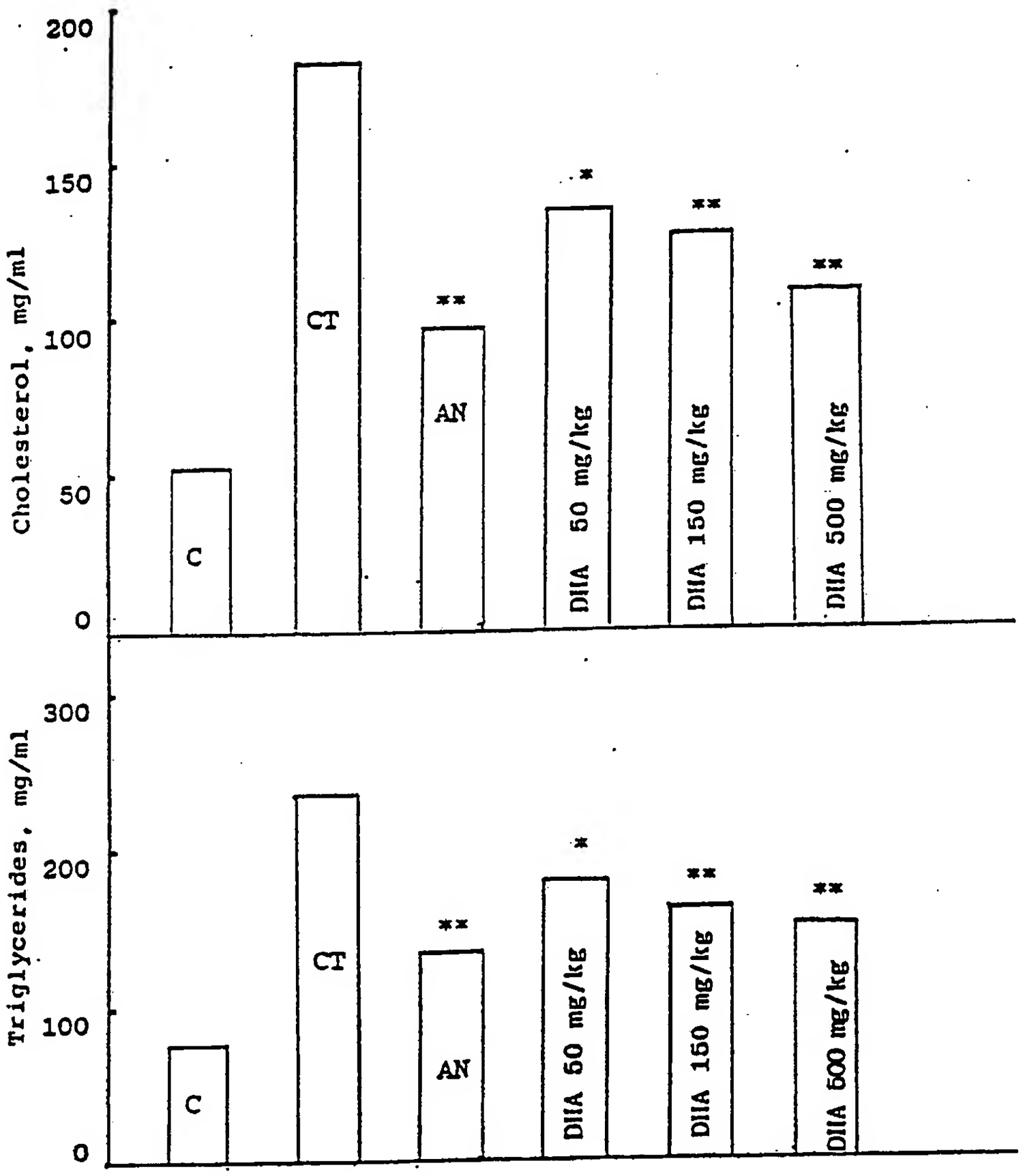
Figure 1 - Effect of the administration of highly purified (96%) DHA on rats affected by hypercholesterolemia and hypertriglyceridemia induced by Nath diet (preliminary data)



** p < 0.01 vs CD

2218904

Figure 2 - Effect of the administration of highly purified (96%) DHA on rats affected by hypercholesterolemia and hypertriglyceridemia induced by WR 1339 (preliminary data)

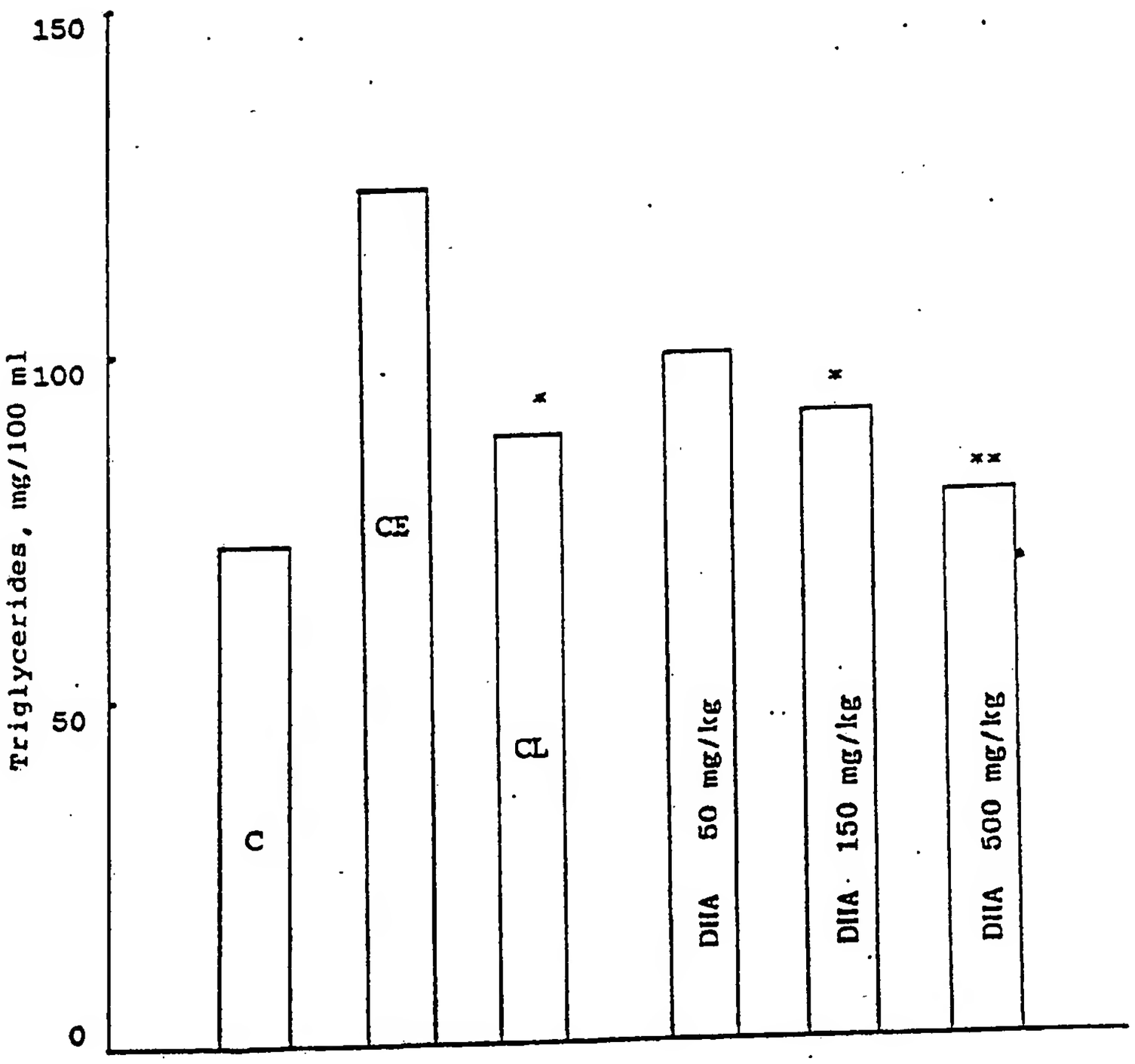


* $p < 0.05$ vs CT

** $p < 0.01$ vs CT

2218904

Figure 3 - Effect of the administration of highly purified (96%) DHA on rats affected by hypertriglyceridemia induced by ethanol (preliminary data)



* p < 0.05 vs CE

** p < 0.01 vs CE

TITLE:

PHARMACEUTICAL COMPOSITION BASED ON HIGH-CONCENTRATION ESTERS OF DOCOSAHEXAENOIC ACID

The present invention relates to a pharmaceutical composition based on high-purity, high-concentration docosahexaenoic acid, in its ester form, useable in a very large number of prophylactic and/or therapeutical application fields.

Docosahexaenoic acid (DHA; C 22:6 n-3) is a highly-unsaturated (six double bonds), long-chain (22 C atoms) fatty acid, whose biological role, together with that of eicosapentaenoic acid (EPA) deserved a considerable interest by the researchers dealing with the relationships between nutrition and health state.

Small amounts of DHA are present in commonly consumed food, and in nature it is exclusively found in animals.

In terrestrial animals, and in particular in mammals, the localization of DHA is practically confined to certain tissues, such as the Central Nervous System (CNS), and in highly specialized structures, such as the synaptic membranes and the retinal cells.

It is interesting to observe that, whilst in lower animal species (marine animals in general, and certain cold water fishes in particular), both DHA and EPA are found, in mammals, and hence in man, DNA is nearly exclusively found, with only traces of EPA being present.

Another difference between mammals and fishes consists in that in upper animals, DHA is nearly exclusively found in ester form in membrane glycerophospholipids, whilst in fishes it also exists in triglyceride form. In order to understand which are the advantages which may be achieved by being able to at last use highly purified DHA as compared to the partial use thereof in association (in fish oils) with EPA, an important foreword is necessary.

In the metabolic path starting from linoleic acid and leading to DHA, through various metabolites, only extremely small amounts of EPA are converted, in short-term studies, into DHA, whilst DHA is converted back into EPA, also in man.

In fact, the intake of DHA in ester form increases to a significant extent both of DHA and EPA levels in plasmatic

phospholipids (Hirai et al., in "Advances in Prostaglandins, Thromboxane and Leukotriene Research", Vol. 17, Eds. Samuelsson B., Paoletti R. and Ramwell P.W., Raven Press, N.Y., page 838, 1978).

This means that DHA, besides a biological activity of its own, can indirectly act, by being reconverted into EPA, as a kind of "pro-drug", thus acquiring biological characteristics typical for EPA as well.

The high titers (up to 90%) and the considerable degree of purity of the EPA/DHA mixtures obtained by means of the process according to the present invention make it possible the pharmaceutical effects to be better pointed out, which derive from the administration of poly-unsaturated acids of omega-3 series, and, in particular, of EPA/DHA.

Having high concentrations of EPA/DHA, on one hand lower-weight, smaller-size pharmaceutical forms can be prepared, which are easier to ingest or administer, and, on the other hand, the number of daily intakes or administrations can be reduced.

The typical characteristics of EPA/DHA products of the present invention make it hence possible a greater therapeutical and formulation advantage to be attained in hyperlipemiae and therewith correlated pathologies, in thromboses, in platelet agglutination, in cardiac infarction, in hypertension, as anticoagulants, in

prevention of atherosclerosis, in cerebral infarction, in lesions and occlusions caused by vasomotor spasms, in diabetes and its complications, in acute and chronic inflammations, in self-immune syndromes, in preventing the side effects at gastroenteric level of non-steroid anti-inflammatory agents, in tumor prevention.

The ratio of EPA concentration to DHA concentration changes according to the natural contents of the organism from which both compounds are extracted (e.g., various fish species, fish oils, crustaceans, sea weeds, and so forth).

The therapeutical properties of mixtures prevailingly containing EPA/DHA, or of mixtures containing, besides other poly-unsaturated fatty acids, also EPA/DHA, have been described in the past in several patents, and in particular: in the treatment of thromboses, in hypercholesterolemiae, in myocardial ischemia (WO 87/03899), in the prevention of arteriosclerosis, in cerebral infarction, in hyperlipemiae, in cardiac infarction (EP-A1-0 228 314), in the prophylaxis of atherosclerosis, as antithrombotic, as antihypertensive (JP 62-091188), in thrombotic pathologies, in platelet agglutination, in self-immune syndromes, in acute and chronic inflammations, in atherosclerosis, cardiac infarction, in venous thromboses, in hyperlipemic states, in hypertension, in lesions and occlusions originated by vasomotor spasms, in diabetes (WO 87/02247), in the

prevention of the side effects of non-steroid anti-inflammatory agents (EP-Al-0 195 570), in the prophylaxis and management of diabetes complications (JP 60-248610), in hypercholesterolemiae, in hypertriglyceridemiae (DE 34 38 630); as anticoagulants, in hypercholesterolemiae (BE 899 184). Furthermore, both EPA and DHA have an influence on the metabolism of poly-unsaturated fatty acids, promoting the formation of products endowed with a high biological activity, i.e., the ecosanoids, which are active in tumor prevention.

Such activities were evidenced by prevailingly using poly-unsaturated fatty acids of omega-3 series, precursors of EPA and DHA (JP 57-187397 and BE 897 806).

The preparations, whose references have been hereinabove cited, are often true mixtures of poly-unsaturated fatty acids prevailingly belonging to omega-3 series, and however, the EPA/DHA concentrations used are always considerably lower than those reached by means of the process according to the present invention.

DHA, a highly unsaturated, long-chain fatty acid, belongs to the series denominated as "omega-3". Differently from what occurs in lower animal species, wherein both eicosahexaenoic acid (EPA) and DHA are present, in man only traces of EPA, and high concentrations of DHA are found.

DHA is present in exclusively esterified form in

membrane glycerophospholipids, and, in particular, in some districts, such as the CNS, in synaptic membranes and in retinal cells.

To the poly-unsaturated fatty acids belonging to omega-3 series, and to EPA, metabolic precursors of DHA, an extremely high number of biological and therapeutical activities have been attributed.

In the metabolic pathway starting from alpha-linoleic acid and leading to DHA, the administration of EPA does not lead, except for small amounts, to conversion to DHA, whilst a portion of administered DHA is converted back into EPA.

In fact, the ingestion of DHA, in ester form, and/or as the free acid, significantly increases both DHA and EPA levels in plasmatic phospholipids (Hiroi at al., 1978).

Thus, DHA, besides performing its own task, would also ensure, by being converted back into EPA, the biological actions typical of EPA.

In prior patents, several therapeutical activities have been claimed for mixtures of poly-unsaturated fatty acids of omega-3 series, to which DHA belongs, and, in particular, therapeutical activities have been claimed in hyperlipemiae and therewith correlated pathologies, in thromboses, in platelet agglutination, in cardiac infarction, in hypertension, as anticoagulants, in atherosclerosis prevention, in cerebral infarction, in lesions and

occlusions caused by vasomotor spasms, in diabetes and its complications, in acute and chronic inflammations, in self-immune syndromes, in preventing the side effects at the gastroenteric level of non-steroid anti-inflammatory agents, and in tumor prevention (WO 87/03899, EP-A1-0 228 314, JP 62-091188, WO 87/02247, EP-A1-0 195 570, JP 60-248610, DE 34 38 630, BE 899 184, JP 57-187397, BE 897 806).

DHA, as a single substance, was evaluated in therapy as a platelet agglutination preventive agent, and an use thereof in the prophylaxis of thrombotic processes was proposed (GB 2,098,065, GB-2,090,529).

In reality, the DHA used in the prior studies does not seem to have been as highly concentrated as DHA used in the present invention, and obtained by means of a process of molecular distillation, disclosed in another patent application by the same Applicant, filed on the same date as of the present application, and having the title: "PROCESS FOR PREPARING HIGH-CONCENTRATION MIXTURES OF POLY-UNSATURATED FATTY ACIDS AND OF THEIR ESTERS FROM OILS OF ANIMAL AND/OR VEGETABLE ORIGIN, AND SO OBTAINED MIXTURES AND THEIR USE FOR PROPHYLACTIC OR THERAPEUTICAL PURPOSES ". By means of such an extraction process, without any chemical of physical treatments, the DHA used in the present invention shows furthermore a purity degree which is considerably higher than of the previously used products.

Furthermore, the process of extraction of the present invention, by means of molecular distillation, without either chemical or physical treatments, characterizes the obtained DHA with a high purity degree, as compared to the previously obtained products.

By means of different experimental models, the activity of highly concentrated (96%) and purified DHA in hyperlipemiae was pointed out. In fact, the administration of DHA reduced, to a meaningful extent, the experimentally induced high levels of cholesterol and triglycerides.

On considering the obtained results, on the basis of the functions which DHA performs inside the organism, and as a consequence of the phenomena observed in various districts when DHA is administered, its characteristics and therapeutical peculiarities can be summarized as follows: in the treatment and prophylaxis of dislipemic diseases and therewith connected pathologies, such as hyperlipoproteinemiae, hypercholesterolemiae, hypertriglyceridemiae, in the alterations of fat metabolism, in damages to vessels caused by cholesterol, in atherosclerosis, in xanthomas, in diabetic retinopathy, in the prevention of thrombus formation, in prevention of aortal and coronary arteriosclerosis, as a coadjuvant in those diseases which may originate manifestations of hyperlipoproteinemiae (diabetes mellitus, hypothyroidism,

uraemia, and so forth), in cardiac infarction, in platelet agglutination, in hypertension, in anticoagulant therapy, in cerebral infarction, in acute and chronic inflammations, in diabetes, in self-immune syndromes, in the prevention of the side effects caused by non-steroid anti-inflammatory agents, in tumor prevention, in retinopathies with visual deficit, in ceroidoses, in the processes relevant to learning and ageing.

DHA acts, through several mechanisms, on some metabolic processes and body districts, favouring a precise pharmacological placing thereof in some pathologies. It is important that preparations of DHA are used, which contain at least 5-10% of alpha-tocopherol, in order to prevent phenomena of peroxidation to the detriment of DHA, which is easily subject to oxidative processes, owing to the high unsaturation level thereof.

1) Action of DHA on metabolism of poly-unsaturated fatty acids.

In nature, several families of fatty acids exist, to each of which compounds belong, which are correlated with one another from a metabolic standpoint.

The three main families of poly-unsaturated acids are constituted by those compounds which belong to n-9, n-6 and n-3 metabolic series. By means of each of said short names, fatty acids are meant, which are endowed with the

characteristic of respectively having the nearest double bond to methyl end (and simultaneously most far away from carboxy end) at a distance of 9, 6 and 3 C atoms.

Inasmuch as the fatty acid molecule portion comprised between the methyl end and the nearest double bond to it is not modified during the metabolic transformations (desaturation and chain extension) which the molecule can undergo, it derives that all compounds formed by metabolic interconversion maintain unchanged this structural characteristic. The respective parent compounds of the mentioned metabolic series of fatty acids are oleic acid, linoleic acid and alpha-linoleic acid, to which DHA belongs.

Linoleic acid and alpha-linoleic acid cannot be synthetized in upper organisms.

Such compounds, which play important biological roles in upper organisms, are hence essential compounds from a nutritional viewpoint, and must be intaken by means of food.

The reactions of metabolic conversion of the above indicated fatty acids into longer-chain, higher-unsaturated compounds take place by means of the activity of desaturation enzymes (desaturases) and chain-extending enzymes (elongases), prevailingly located at liver level (reticuloendoplasmatic system).

The following considerations are important, as relates to the metabolism of the various series of poly-unsaturated fatty acids.

a) Regulation steps for such reactions axist, and, in particular, the desaturation reactions are limiting steps.

b) The speeds of conversion of the precursors of the three series of fatty acids are very different from one another; such a speed is much higher for alpha-linoleic acid 18:3 (n-3 series), much lower for linoleic acid (18:2, n-6), and minimum for oleic acid.

c) A competitive antagonism exists in the metabolism of the three series of poly-unsaturated fatty acids, with the consequent inhibition, by the larger-affinity acids, of the metabolic conversion of the lower-affinity acids. On the contrary, the lack in the diet of acids with a high affinity for the enzymatic systems (e.g., the lack in essential linoleic and alpha-linoleic acids) unblocks the conversion of lower-affinity acids (oleic acid).

Therefore, in case of deficiencies of essential fatty acids, a conversion of oleic acid up to eicosatrienoic acid, C 20:3 n-9 takes place.

d) As a consequence of the metabolic interactions between the various unsaturated fatty acids (n-3, n-6, n-9)

supplied with the diet, it happens that the mutual ratio thereof conditions the metabolic conversion of C_{18}-compounds belonging to the various series into more-unsaturated, longer-chain compounds, and, consequently, their incorporation with plasmatic and tissular lipids.

The administration of poly-unsaturated acids of n-3 series, such as DHA, is hence very important as to their metabolic use, and their incorporation with the tissues.

In fact, it is evident that the administration, e.g., of DHA, will cause an incorporation of such compound with the cellular lipids, which will depend on several factors, such as the relative levels of n-6 acids in the diet, besides the relative affinity of DHA, as compared to that of n-6 acids, for the various phospholipidic pools in different cellular types.

2) Action of DHA on vascular district, on atherosclerosis, platelet functions and thrombus formation.

As a consequence of what reported above on the influence of DHA on the metabolism of unsaturated fatty acids, the role is important which it may play on C_{20} poly-unsaturated fatty acids, precursors of products endowed with a high biological activity, viz., eicosanoids.

Eicosanoids are substances prevailingly deriving by enzymatic oxygenation from arachidonic acid (AA), through

the following two main routes: cyclooxygenase, leading to the formation of prostaglandins, prostacycline and thromboxane, and lipoxygenase, leading to the formation of hydroxyacids and leukotrienes.

Some specific eicosanoids may take an action in the mechanisms which regulate important functions, and which constitute the basis for some processes, such as in the formation of thrombi, and in vascular district (activation of production of I_3 prostacycline, or PGI_3, instead of PGI_2).

Through the formation of the specific eicosanoids and the consequent production of inactive TxA_3, DHA also acts on platelet agglutination (Rao G.H.R. et al., Biochem. Biophys. Res. Comm., 117, 549, 1983). However, the effects on some systems of the administration of purified DHA are rather different from the effects exhibited by EPA.

In fact, although after DHA administration a platelet agglutination preventive activity takes place, no particular interferences with the metabolism of AA were observed, differently from what occurs when EPA is administered (Hirai et al., in "Advances in Prostaglandins, Thromboxane and Leukotriene Research", Vol. 17, Eds. Samuelsson B., Paoletti R. and Ramwell P.W., Raven Press, N.Y., page 838, 1978). Such data

suggests that DHA incorporated with platelet lipids is neither easily released, nor, consequently, converted into other compounds.

Furthermore, DHA, according to the studies by Talesnik and Carleton (Talesnik J, and Carleton Hsia J., Eur. J. Pharmacol., 80, 255, 1982), inhibits, at a coronaric level, the vasoconstriction induced by AA.

It derives therefrom that DHA, through direct conversion, or reconversion into EPA with intervention on the metabolism of C_{20}-fatty acids, can lead to the formation of specific eicosanoids, with the possibility of acting, at a preventive level, or at a therapeutical level, as an antithrombotic, in extending the bleeding times, as a coronary vasodilator, and as a platelet agglutination inhibitor.

3) Action of DHA on immunity system and in inflammation

The fatty acids belonging to n-3 series, to which DHA belongs, play an important role in varying the immunity and inflammatory responses, through the modifications in the cellular poly-unsaturated fatty acids, with consequent changes in the synthesis of prostaglandins and leukotrienes, in the cells engaged in the immunity and inflammatory responses (leukocytes, monocytes, T and B lymphocytes).

DHA performs an action in the inflammatory process on the

synthesis of prostaglandins through the competitive inhibition of the conversion of arachidonate into PGE_2, as it occurs for some non-steroid anti-inflammatory agents, such as indomethacin (Corey E.J. et al., Proc. Natl. Acad. Sci., 80, 3581, 1983).

Furthermore, various other processes of cellular activation can be modified by changes in the poly-unsaturated fatty acids in structural membrane lipids, as a consequence of the administration of fatty acids of n-3 series. Among these, an important role has to be assigned to the mobilization of intracellular Ca after stimulation, and to the generation of inositol phosphates from membrane phosphoinositide pools.

4) Action of DHA on visual functionality, ceroidosis, in learning and ageing processes

High concentrations of DHA are contained in retina and in synaptic terminations.

The depletion of DHA in such structures, obtained by means of dietetic manipulations in laboratory animals, causes visual malfunctions. (Tinoco J. et al., Biochim. Biophys. Acta, 486, 575, 1977).

Therefore, the availability of highly purified DHA in the treatment of those pathologies correlated with a decrease in DHA concentrations in retinal glycerophospholipids seems to be highly interesting from a therapeutical

viewpoint.

DHA depletion causes alterations in behaviour, with learning deficit. In fact, as Lamptey and Walker were able to observe (Lamptey M.S. and Walker B.L., J. Nutr., 106, 86, 1976) the learning capability shown by rats submitted to diets with different added amounts of n-3 acids was directly proportional to cerebral levels of 22:6 (DHA). Particularly interesting is also the correlation between ceroidosis and DHA concentration.

Ceroidosis is a pathology wherein a lipofucsinic pigment of brown colour (ceroid) can deposit inside cells of smooth-muscles of digestive tube, in liver, in muscles and in CNS.

Pullarkat et coworkers (Pullarkat R.K. et al., Neuropädiatrie, 9, 127, 1978) evidenced that in juvenile neuronal ceroidosis a correlation exists between the decrease in leukocytic DHA, found in all examined patients, and the seriousness of the disease.

Furthermore, the same Authors observed, in a prior study, a decrease in DHA content in phosphatidylserine present in cerebral gray in adult and juvenile forms of neuronal ceroidosis.

The supply of exogenous, highly-purified DHA may be regarded as a valuable therapeutical means in the evolution of such a pathology.

As reported in the foreword, DHA is found in upper animals in ester form in membrane glycerophospholipids. Among main tissular glycerophospholipids (phosphatidyl-choline, PC or lecithin; phosphatidyl-ethanolamine, PE; phosphatidylserine, PS; and phosphatidyl-inositol, PI), and, in particular, PE and PS at a cerebral level, contain relatively high levels of DHA. The relative concentrations of DHA, relatively to total fatty acids, at the level of membranes of preparations of synaptosomes may reach values as high as 20%, with a ratio of >1 relatively to AA, the main poly-unsaturated acid present in other tissues. Said two phospholipids, PE and PS, are localized on the inner cellular membrane surface, and therefore the high DHA levels in such phospholipids suggest that such a fatty acid may be involved in some functions inside the cell. It is also interesting to observe that PC and PI, at the cerebral level, either contain very low DHA levels, the first one, or do not contain any DHA at all, the second one (Galli C. et al., in "Advances in Prostaglandins and Thromboxane Research", vol. 4, Eds. Coceani F. and Olley P.M., Raven Press, N.Y., page 181, 1978).

In the same work, the particular abundance of 22:6 (DHA) in phospholipids of synaptosomial membranes is observed. The administration of a diet containing an oil rich in n-

3 acids, and in particular of DHA, such as a fish oil, causes a sharp increase of this poly-unsaturated acid in membrane lipids.

Also in man (White H.B. et al., J. Neurochem. 18, 1337, 1971) decrease at cerebral level during the course of ageing.

In lipids isolated from cerebral synaptosomes of aged rats, a significant decrease in DHA is observed as compared to the values which are observed in adult rats.

Studies on rat also demonstrated that the capability of incorporating DHA administered by means of the diet, with cerebral lipids, considerably decreases during ageing (Eddy D.E., Harman D., J. Am. Ger. Soc. 25, 220, 1977). These Authors postulated that such a decrease is due to an increase in lipid peroxidation at the cerebral level during the course of ageing.

Therefore, the possibility of being able to use concentrated DHA in all those complex mechanisms which lead to the learning and ageing process seems to be of particular moment.

Furthermore, of considerable interest appears to be the fact that several proprietary medicines used in medical practice contain cerebral phospholipids, the natural seat of DHA, with the following operating mechanisms and indications.

Phosphatidylserine: influence on the parameters of cerebral metabolism, altered during ageing. Therapeutical applications in chronic cerebral psycho-organic syndromes and valuable use in therewith correlated symptoms (lack in memory - confusion - poor attention and concentration, emotional lability, irritability, depressed mood, anxiety).

Diencephalic phospholipids: The liposomes of hypotalamus phospholipids are capable of activating the hypotalamus metabolism, increasing dopamine turnover, the activity of tyrosine-hydroxylase and of adenylcyclase, with a consequent increase in cyclic AMP. This effect reflects itself in particular on the functionality of hypotalamus-hypophysis axis. Application as a coadjuvant in cerebral metabolic alterations consequent to neuroendocrine disorders such as depressive syndromes, anxious-depressive statuses occurring during developmental age, in climacteric syndrome and in hypoprolactinemiae.

Cerebral phospholipids: are capable of activating the neuronal metabolism, normalizing the enzymatic activities of membranes, increasing the neurotransmitters turnover. Therapeutical application in neurologic syndromes such as arteriosclerotic cerebrovascular pathologies, involution syndromes, parkinsonian syndromes, cranio-encephalic traumatic lesions and psychosomatic hypoevolutism,

as well as in all pathologies connected with altered statuses of CNS metabolism.

5) Action of DHA on cardiac functionality

The interest in the favourable effects of n-3 acids on various biologic parameters above all of cardiovascular district arouse nearly ten years ago.

The key observations which played a determining role in promoting this interest were some epidemiological studies carried out on populations (in particular the Eskimos) consuming very large amounts of fish, very rich in DHA, which evidenced an extremely low incidence of cardiovascular pathologies, notwithstanding the high contents of fats in their diet.

On rat, it was observed that the metabolism and the function at the cardiac level, in animals submitted to diets with different compositions as for fatty acids, are correlated with the contents of DHA in cardiac phospholipids (Gudbjarnason S. et al., Acta Biol. Med. Germ., vol. 37, 777, 1978).

In fact, the exposure to catecholamine-stress involved the replacement of linoleic acid by DHA in cardiac lipids, whilst cardiac frequency in groups of animals submitted to different diets was proportional to the DHA contents of the same lipidic fractions.

The therapeutical properties and prevention capabilities

of DHA, in certain cardiac diseases, are probably also bound, besides a direct action, to the different activities thereof in such different compartments as anti-atherosclerotic activity, antithrombotic activity, hypolipemic activity and platelet agglutination preventive activity.

6) Action of DHA on hyperlipemiae

Hyperlipoproteinemic pathology is a condition wherein the concentration of cholesterol or of the lipoprotein-bearing triglycerides in plasma exceeds the normal physiologic limits.

At present, the percentage of population affected by high values of plasmatic concentration of cholesterol and/or triglycerides is estimated to be around 95% of total. These limits vary according to age and sex.

Hyperlipoproteinemiae can be subdivided into primary and secondary hyperlipoproteinemiae.

Primary hyperlipoproteinemiae may be subdivided into two main groups: the monogenic primary hyperlipoproteinemiae (of genetic origin) and the polygenic primary hyperlipoproteinemiae (probably in already predisposed individuals, with the addition of incorrect diets and obesity).

The secondary hyperlipoproteinemiae evidence themselves as complications of metabolic disturbances in some

pathologies, as diabetes mellitus, hypothyroidism, uremia, overdrinking, or as secondary effects in the use of oral contraceptives in subjects genetically prone to hypertriglyceridemia.

It is ascertained by now on scientific grounds that high concentrations of lipoproteins accelerate the development of arteriosclerosis, which may subsequently cause such irreparable damages as thrombosis and cardiac infarction. According to computations, in the U.S.A. approximately half deceases are likely to be due to such events.

With the evolution of industrialized civilization and with the relevant increase in social welfare, people, especially over past decades, started to overindulge in food, both as regards the amounts, and, from the dietetic-nutritional viewpoint, the quality.

In fact, the intake hase progressively increased of food rich in cholesterol and saturated fatty acids.

This is one of the main causes of the increase in dislipemic pathologies.

The first therapeutical aid for all hyperlipoproteinemiae is the use of a diet which maintains a normal body weight and reduces the concentrations of lipids in blood.

The elimination from the diet of saturated animal oils, to be replaced by poly-unsaturated vegetable oils, is a priority condition for lipidic illnesses to show a

positive evolution.

Due to this reason, we took into consideration the possibility that DHA, a poly-unsaturated long-chain fatty acid, may perform an action in dislipemic pathology.

Therefore, some preliminary pharmacologic tests were developed, in order to evidence the possible activity of DHA, by oral way, in test animals.

In all tests, Sprague-Dawley albino rats were used.

Esterified highly-concentrated DHA, having a titer of 96%, was used.

A group of animals per each test were treated with clofibrate or nicotinic acid, well-known molecules endowed with hypolipemic activity, in order to verify the experimental validity.

All animals, at sacrifice time, were under fasting conditions.

A) Activity of DHA in hyperlipemia induced by Nath diet.

Nath diet is a diet above all rich in cholesterol and cholic acid and, when intaken for a 4-5 weeks time, causes hyperlipemia in the animal.

Male rats of an initial weight of approximately 80-100 grams were used.

The animals were subdivied into 6 experimental groups:

1) controls (C);

2) controls + Nath diet (CD);

3) clofibrate, 300 mg/kg daily + Nath diet (CL);

4) DHA, 50 mg/kg daily + Nath diet;

5) DHA, 150 mg/kg daily + Nath diet;

6) DHA, 500 mg/kg daily + Nath diet.

The test lasted 5 weeks, during which the general conditions of the animals (growth, food consumption, etc.) were monitored.

At test end, the animals were sacrificed under fasting conditions, in order to evaluate the parameters (total cholesterol, triglycerides)

The results obtained confirm that the administrations of highly concentrated DHA inhibit the hyperlipemic effects experimentally induced by the nourishing with the Nath diet (Figure 1).

B) Activity of DHA on hyperlipemia induced by Triton WR 1339.

Triton WR 1339 or Tyloxapol is a substance capable of increasing the hematic levels of triglycerides and cholesterol in the laboratory animal.

Male rats of approximately 200 g of initial weight were used.

The animals were subdivided into 6 experimental groups:

1) controls (C);

2) controls + Triton (CT);

3) nicotininc acid, 500 mg/kg daily + Triton (AN);

4) DHA, 50 mg/kg daily + Triton;

5) DHA, 150 mg/kg daily + Triton;

6) DHA, 500 mg/kg daily + Triton.

The animals, before receiving Triton WR 1339, were pre-treated for a 2-days period with nicotininc acid, DHA or with the carrier only.

Eighteen hours after the treatment with Triton WR 1339, the animals, under fasting conditions, were sacrificed for the evaluation of the hematic levels of total cholesterol and triglycerides.

In this test too, the administration of DHA evidenced capability of this compound of decreasing the levels of the plasmatic lipids altered by the treatment with Triton WR 1339 (Figure 2).

C) Activity of DHA in hypertriglyceridemia induced by ethanol

The subacute administration of ethanol causes, in rat, a condition of hypertriglyceridemia.

For this test, male rats of an initial weight of approximately 200 grams were used.

The animal were subdivided into six test groups:

1) controls (C);

2) controls + ethanol (CE);

3) clofibrate, 200 mg/kg daily + ethanol (CL);

4) DHA, 50 mg/kg daily + ethanol;

5) DHA, 150 mg/kg daily + ethanol;

6) DHA, 500 mg/kg daily + ethanol.

Oral administrations of ethanol ad libitum in solution at 10%, alternating with administrations of 0.5 g/rat, induce hypertriglyceridemia in rat.

Since the day prior to the first intake of ethanol, the animals were treated with the substances under test. On the 4th day, 2 hours after the last treatment with ethanol, the animals were sacrificed in order to determine the triglycerides.

In this test, very reassuring results were obtained. In fact, the oral administration of high-concentration DHA significantly inhibited hypertrigliceridemia induced by ethanol (Figure 3).

Summing up, on considering the results obtained in the experimental tests, the use of high-titer DHA (96%) in the therapy of hyperlipemic pathologies and in the therewith correlated pathologies can be regarded as at all justified.

Claims

1. Pharmaceutical composition containing docosahexaenoic acid (DHA) as the active substance, characterized in that the active substance is contained in a highly purified, high-concentration ester form, with the addition of tocopherol as an antioxidant.

2. Pharmaceutical composition according to claim 1, characterized in that DHA is present in its ethyl ester form.

3. Pharmaceutical composition according to claim 2, characterized in that DHA is present at a total concentration of at least 90%.

4. Pharmaceutical composition according to claim 1, characterized in that alpha-tocopherol is added in an amount comprised within the range of from 5 to 10% by weight.

5. Pharnaceutical composition according to claim 1, characterized in that the purification of DHA is obtained by measn of molecular distillation.

6. Use for prophylactic or therapeutical purposes of highly purified docosahexaenoic acid (DHA), prepared in its esterified form, and with a suitable addition of alpha-tocopherol (in an amount comprised within the range of from 5 to 10%), as claimed in claims 1-5, in the following alterations connected with the unbalance of the lipoproteinemic picture and with the therewith correlated

pathologies:

- in hyperlipoproteinemiae;
- in hypercholesterolemiae;
- in hypertriglyceridemiae;
- in alterations of lipidic metabolism;
- in vessel damages caused by cholesterol and saturated fatty acids;
- in atherosclerosis;
- in xanthomas;
- in diabetic retinopathy;
- in thrombus formation prevention;
- in prevention of aortic and coronary atherosclerosis;
- in platelet agglutination;
- as a coadjuvant in those diseases which can originate hyperlipoproteinemic manifestations, such as, e.g., diabetes mellitus, hypothyroidism, uremia, and so forth.

7. Use for prophylactic or therapeutical purposes of highly purified docosahexaenoic acid (DHA), prepared in its esterified form, and with a suitable addition of alpha-tocopherol (in an amount comprised within the range of from 5 to 10%), as claimed in claims 1-5, in the following indications:

- in the prevention of cardiovascular diseases;
- in cardiac hyperexcitability;
- in vasal hypertension;

- in coronaric vasoconstriction (angina pectoris);
- in inflammatory diseases;
- in diseases requiring a larger supply of immunity stimuli;
- in the pathologies wherein a deficiency of DHA occurs, such as the retinopathies with visual deficits, in processes connected with learning, in ageing and in ceroidoses.

8. Pharmaceutical composition as claimed in Claim 1, substantially as described in any of the examples disclosed herein.